Appl Microbiol Biotechnol (2012) 93:903–916
DOI 10.1007/s00253-011-3753-x

MINI-REVIEW

Beneficial effects of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and its fermented products

Shen-Shih Chiang · Tzu-Ming Pan

Received: 19 September 2011 / Revised: 7 November 2011 / Accepted: 21 November 2011 / Published online: 9 December 2011

Abstract It is well-known that probiotics have a number of beneficial health effects in humans and animals, including the reduction of symptoms in lactose intolerance and enhancement of the bioavailability of nutrients. Probiotics have showed to possess antimutagenic, anticarcinogenic and hypocholesterolemic properties. Further, they were also observed to have antagonistic actions against intestinal and food-borne pathogens, to decrease the prevalence of allergies in susceptible individuals and to have immunomodulatory effects. Typically, the bacteria colonise the intestinal tract first and then reinforce the host defence systems by inducing a generalised mucosal immune response, balanced T-helper cell response, self-limited inflammatory response and secretion of polymeric IgA. Scientific reports showed that the Taiwan native lactic acid bacterium from newborn infant faeces identified as *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and its fermented products proved to be effective for the management of blood cholesterol and pressure, prevention of gastric mucosal lesion development, immunomodulation and alleviation of allergies, anti-osteoporosis and inhibition the fat tissue accumulation. This review article describes that the beneficial effects of this *Lactobacillus* strains and derivative products may be suitable for human and animals.

S.-S. Chiang
Department of Food Science and Biotechnology,
College of Agriculture and Natural Resources,
National Chung Hsing University,
250, Kuo Kuang Rd.,
Taichung, Taiwan, Republic of China

T.-M. Pan (✉)
Department of Biochemical Science and Technology,
College of Life Science, National Taiwan University,
1, Sec. 4, Roosevelt Road,
Taipei 10617, Taiwan, Republic of China
e-mail: tmpan@ntu.edu.tw

Keywords *Lactobacillus paracasei* subsp. *paracasei* NTU 101 · Hyperlipidemia · Hypertension · Gastric mucosal lesion · Osteoporosis · Obesity

Introduction

Lactic acid bacteria (LAB) collectively represent a major part of the commensal microbial flora of the human gastrointestinal tract and are frequently used as probiotics either singly or in combination for fermentation of food products. *Lactobacillus*, *Streptococcus* and *Bifidobacterium* are the most commonly used (Fooks et al. 1999). Probiotics are available in different forms, including foods in a fermented state, and in the form of pills mainly as capsules or microencapsulated forms. By considering the many types of potential fermentation substrates and conditions, LAB strains may be regarded as functional performance bacteria and are expected to be used in the development of new food-based probiotics in the future. Probiotics not only acts on the large intestine by affecting the intestinal flora but also affects other organs by modulating the immunological parameters and intestinal permeability and by producing bioactive or regulatory metabolites (de Vrese and Schrezenmeir 2008). Dietary supplementation with live beneficial bacteria promotes health effects and reduces the risk of various diseases (Ahrne et al. 1998).

The probiotic market in the USA was estimated to be close to $1.5 billion in 2010. Total production of sour cream and yogurt was 5.4 billion lb at 116 processing plants in the USA in 2010 (USDA 2011). Increased production has been observed among the dairy-type and non-dairy probiotic foods, such as fermented meats as well as vegetable and fruit juices. Considering the wide range of fermentable substrates that can be used to obtain different products under different conditions by LAB strains, it is expected that development of new food-based probiotics will continue in the future (Holzapfel 2006).

Lactobacilli are gram-positive, non-spore forming, rod-shaped, anaerobic bacteria. Many *Lactobacillus* strains are used in food fermentation and are typically used in the dairy industry to produce cheese, yogurt and other fermented milk products (Schmid et al. 2006). They have complex nutritional requirements and are found in a variety of habitats, such as human and animal mucosal membranes, on material of plant origin and in sewage as well as in fermented milk products and spoiling food (de Vrese and Schrezenmeir 2008). They are important for enhancing immunity, maintenance of the intestinal microbial balance and prevention of gastrointestinal infection.

A lactic acid bacterium isolated from native newborn infant faeces in Taiwan was characterised, and the isolates were identified as *Lactobacillus paracasei* subsp. *paracasei* NTU 101. This strain showed good survival at low pH, tolerance against high bile concentrations and the ability to reduce serum cholesterol in vitro (Pan et al. 2002). Furthermore, a milk–soymilk mixture was fermented by *L. paracasei* subsp. *paracasei* NTU 101 and *Bifidobacterium longum* BCRC11847. Based on the sensory evaluation results, supplementation with 5% *Lycium chinense* Miller juice was found to improve the overall acceptability of the fermented milk–soymilk mixture. The pH and titratable acidity in the fermented beverage was slightly altered, and the quantities of cells of *L. paracasei* subsp. *paracasei* NTU101 and *B. longum* BCRC11847 were maintained at 1.2×10^9 and 6.3×10^8 CFU/mL after 14 days of storage (Lin et al. 2004). Many studies have also proved that the beneficial effects of *L. paracasei* subsp. *paracasei* NTU 101 and its fermented foods were clearly well defined. This article is summarised and compared with different effects among lactic acid bacteria strains.

Hypocholesterolemic effect

Hypercholesterolemia increases the risk of cardiovascular diseases (CVDs) such as atherosclerosis, coronary heart disease and stroke. An estimated 6.15 million people died from CVDs in 2008. This represents 10.8% of all global deaths (WHO 2011). Low levels of high-density lipoprotein cholesterol, high levels of low-density lipoprotein cholesterol (LDL-C) and oxidation of LDL are major risk factors and initiators of atherosclerosis. Xie et al. (2011) found that Sprague–Dawley rats fed a high-cholesterol diet experienced reduced levels of serum cholesterol, LDL-C and triglyceride (TG) after supplementation with *Lactobacillus plantarum* 9-41-A and *Lactobacillus fermentum* M1-16. These two strains also improved the intestinal microbial balance and the hypocholesterolemic effect. Wistar albino rats were fed 20% fresh or oxidised soybean oil supplemented with 5% lyophilised culture or fermented milk prepared using *Lactobacillus casei* subsp. *casei* for a period of 90 days (Kapila and Sinha 2006). The plasma cholesterol levels and thiobarbituric acid reactive substances (TBARS) in the LDL fraction were lower in the group that was fed fermented milk and lyophilised culture than the control group that was fed skim milk.

In an earlier study, Syrian hamsters were fed a high-cholesterol diet (5 g/kg diet) to induce hypercholesterolemia. Milk fermented by *L. paracasei* subsp. *paracasei* NTU 101, *L. plantarum* NTU 102 and *Lactobacillus acidophilus* BCRC 17010 was fed for 8 weeks. The levels of serum and liver total cholesterol were also significantly reduced by 23.5–30.1% and 13.4–17.7%, respectively. These results showed that the hypocholesterolemic effect of local *Lactobacillus* strains may be attributed to their ability to lower serum and liver total cholesterol levels (Chiu et al. 2006).

In addition, mixtures of milk–soymilk and milk–soymilk supplemented with *Momordica charantia* were fermented by lactic acid bacteria, and the effect on atherosclerosis in hyperlipidemic hamsters was investigated (Tsai et al. 2009). The hamsters that were fed *L. paracasei* subsp. *paracasei* NTU 101 fermented milk–soymilk with and without *M. charantia* experienced significantly decreased serum cholesterol levels. Furthermore, atherosclerotic plaques in the aorta were decreased by 69.0% relative to the group of hamsters that were fed a high-cholesterol diet (H) (Fig. 1). It was concluded that the anti-atherosclerotic activity of NTU 101 fermented milk–soymilk is due to an increase in the total antioxidant status activity of the blood and decrease in the quantity of TBARS.

In high-cholesterol diet model, levels of cholesterol and TBARS were elevated in rats or hamsters, while feeding with the lactobacilli strains or fermented milks was effective in reducing serum cholesterol, LDL-C and TG concentrations. The mechanisms of lactic acid bacteria in altering the serum cholesterol from these above mentioned results may propose by directly absorbing and assimilation cholesterol into the cell membrane (Noh et al. 1997) and decreasing HMG-CoA reductase activity and cholesterol micelle formation in vivo by metabolites of lactic acid bacteria like short chain fatty acids (Fukushima and Nakano 1996). We could suppose that *L. paracasei* subsp. *paracasei* NTU 101-fermented milk–soymilk is effective in preventing and retarding hyperlipidemia-induced oxidative stress and atherosclerosis.

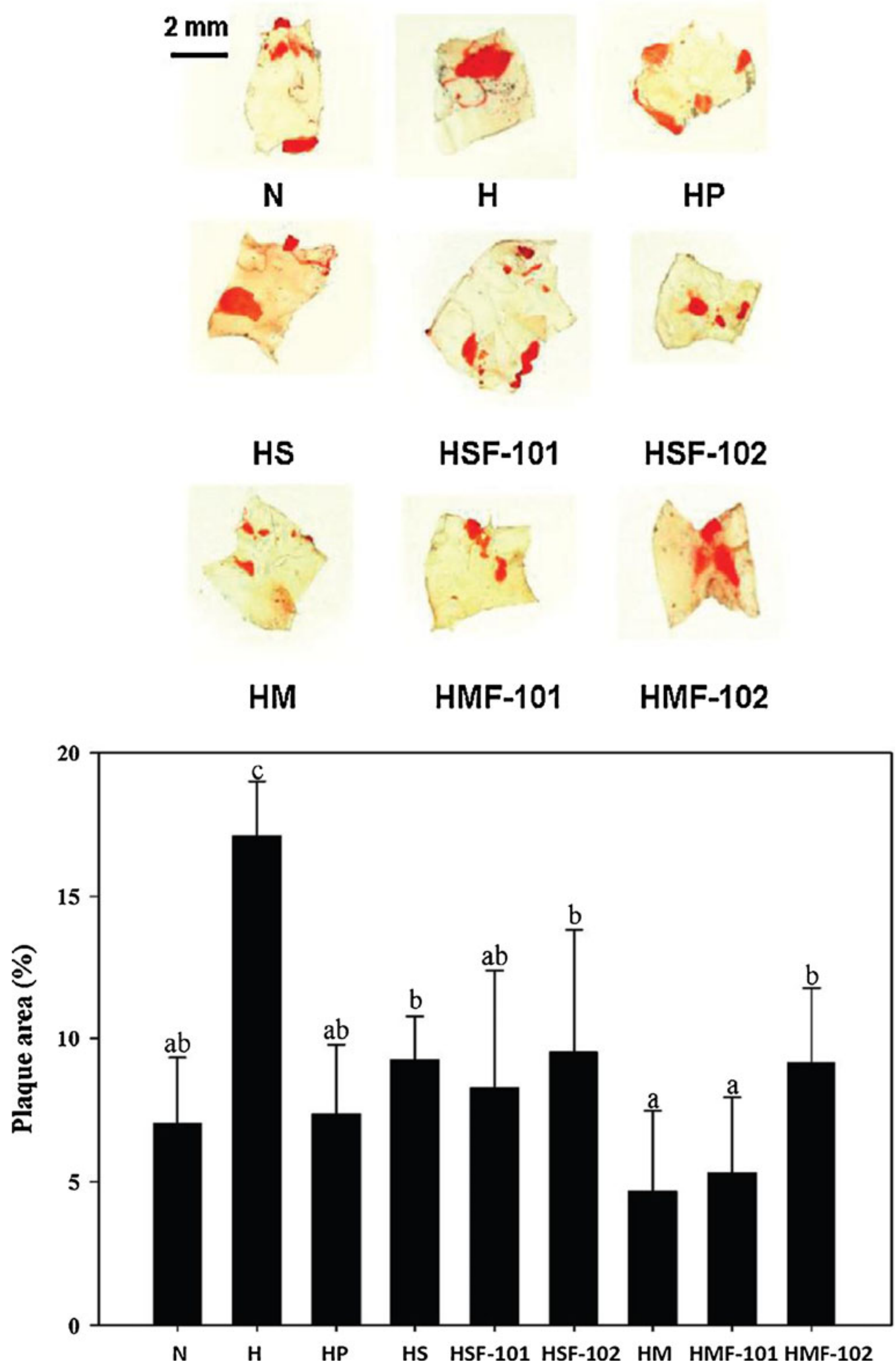


Fig. 1 Effect of milk–soymilk and fermented milk–soymilk on atherosclerotic plaque in the thoracic aorta of hyperlipidemic hamsters. *Top* atherosclerotic plaque presented as the *red dye* in the graph. *Bottom* Proportion of the area of atherosclerotic plaque in the aorta. The whole surface area of thoracic aorta was stained by Sudan IV and photographed using digital camera. The aortic surface area and its stained plaque area (*red dye*) were selected and quantitated by the Posterize program of Photoshop 7.0 software (Adobe Systems Incorporation, San Jose, CA, USA). The selected pixel of plaque area and whole aorta was used to calculate the percent area of the aortic plaque. *N* normal diet (0% cholesterol), *H* high-cholesterol diet, *HP* probucol and high-cholesterol diet, *HS* milk–soymilk and high-cholesterol diet, *HSF-101* milk–soymilk fermented by *L. paracasei* subsp. *paracasei* NTU 101 and high-cholesterol diet, *HSF-102* milk–soymilk fermented by *L. plantarum* NTU 102 and high-cholesterol diet, *HM* milk–soymilk supplement with *M. charantia* and high-cholesterol diet, *HMF-101* milk–soymilk supplement with *M. charantia* fermented by *L. paracasei* subsp. *paracasei* NTU 101 and high-cholesterol diet, *HMF-102* milk–soymilk supplement with *M. charantia* fermented by *L. plantarum* NTU 102 and high-cholesterol diet. Data are presented as mean±SD (n=8), and *numbers with a different letter* were significantly different (p<0.05) from each other (Tsai et al. 2009)

Antihypertensive effect

Hypertension is another risk factor for CVDs. Angiotensin-converting enzyme (ACE; EC 3.4.15.1) mediates blood plasma, interstitial fluid volume and arterial vasoconstriction in the body's renin–angiotensin system (Imig 2004). ACE inhibitors have beneficial effects in hypertension by inhibition of the production of the vasoconstrictor angiotensin II and degradation of the vasodilator bradykinin (Seppo et al. 2003).

LAB-fermented products may prevent hypertension via substances such as γ-aminobutyric acid (GABA) and angiotensin I-converting enzyme inhibitors (ACEIs). In 2002, Fuglsang et al. (2002) reported that feeding of milk fermented with *Lactobacillus helveticus* CHCC637 and CHCC641 to rats produces inhibition of angiotensin II conversion, a drop in blood pressure and changes in heart rate. Milk fermented with *L. helveticus* strains can produce ACEI biogenic tripeptides like Val-Pro-Pro and Ile-Pro-Pro as antihypertensive ingredients in spontaneously hypertensive rats (SHR) (Yamamoto et al. 1999; Takano 2002; Seppo et al. 2003) and hypertensive subjects (Hata et al. 1996; Jauhiainen et al. 2010).

It was previously found that milk fermented with *L. paracasei* subsp. *paracasei* NTU 101 (101FM) has ACEI activity (85 mU/mL). This study aimed to investigate the antihypertensive effects of 101FM orally administered to SHRs. Eight hours after a single oral administration or after 8 weeks of weekly (chronic) administration, 101FM caused a significant decrease in systolic and diastolic blood pressure in the SHRs (Fig. 2). Microscopic examination of aortic tissue demonstrated that 101FM reduces the disorganization of the media layer (Fig. 3). These findings suggest that orally administered 101FM has antihypertensive effects, possibly via ACEI activity, in SHRs (Liu et al. 2011b).

All these results recommended that milk fermented by lactic acid bacteria could enhance the content of bioactive peptides (Yamamoto et al. 1999; Takano 2002; Seppo et al. 2003; Hata et al. 1996; Jauhiainen et al. 2010; Liu et al. 2011b) or aglycone isoflavones (Yeo and Liong 2010) to reduce the blood pressure in animal model. The mechanism of antihypertensive effects from milk peptides is mainly focused on the inhibition of angiotensin-converting enzyme. However, milk peptides may also have special mechanisms to lower blood pressure by opioid-like activities, mineral-binding and antithrombotic properties (Jauhiainen and Korpela 2007).

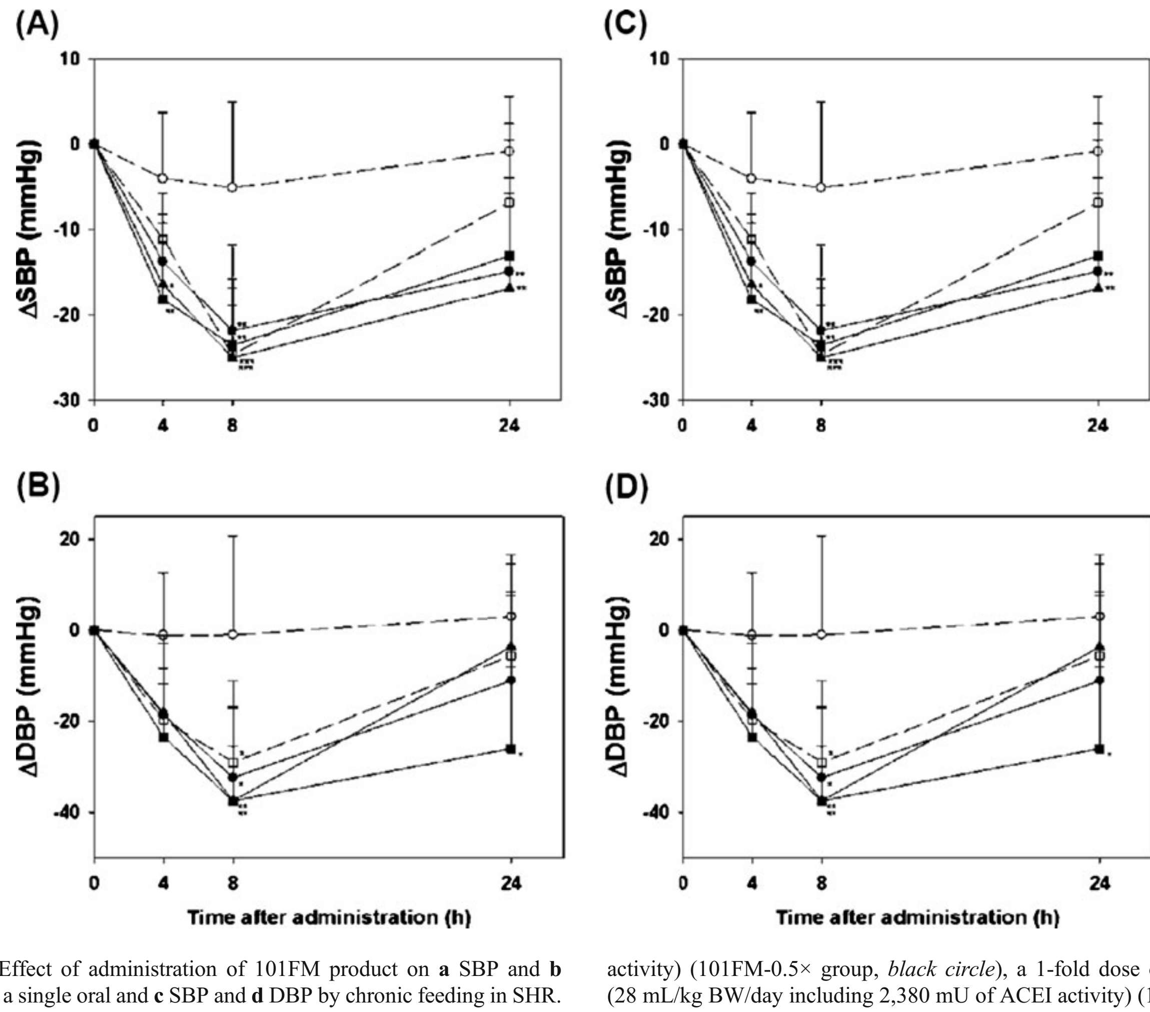


Fig. 2 Effect of administration of 101FM product on **a** SBP and **b** DBP by a single oral and **c** SBP and **d** DBP by chronic feeding in SHR. One group of the SHRs was fed a normal diet without the administration of test materials (control group, *white circle*). The other SHRs were administered a 1-fold dose of Calpis (15 mL/kg/day including 2,380 mU of ACEI activity) (Calpis group, *white square*), a 0.5-fold dose of 101FM (14 mL/kg BW/day including 1,190 mU of ACEI activity) (101FM-0.5× group, *black circle*), a 1-fold dose of 101FM (28 mL/kg BW/day including 2,380 mU of ACEI activity) (101FM-1× group, *black square*) and a 2-fold dose of 101FM (56 mL/kg BW/day including 4,760 mU of ACEI activity) (101FM-2× group, *black triangle*). *$p<0.05$, p estimated by one-way ANOVA and versus the control group ($n=8$) (Liu et al. 2011b)

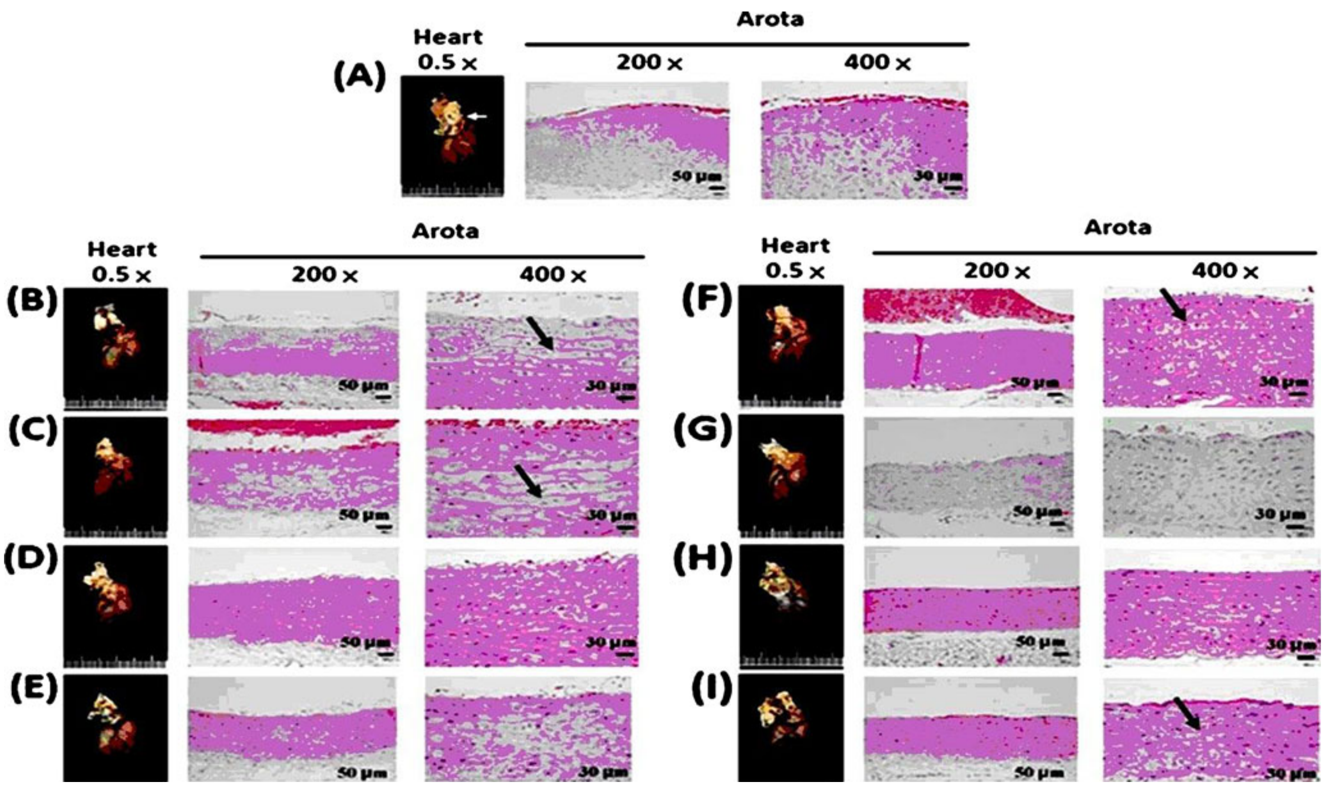


Fig. 3 Heart appearance and microscopic examination (×200 and ×400) of aorta biopsy on experimental SHRs: **a** control group, **b** Calpis group, **c** GABA group, **d** 101FM-0.5× group, **e** 101FM-1× group, **f** 101FM-2× group, **g** 102FM-0.5× group, **h** 102FM-1× group, **i** 102FM-5× group (the *arrow* points out the location of difference) (Liu et al. 2011b)

Beneficial effects on immunomodulating activity

Recently, immune-mediated and gut-related health problems have been recognised as global major progressive diseases. Many rigorous studies have suggested that emerging nutritional strategies including probiotics diets may be able to reduce these host-related immune diseases and modulate the intestinal microbiota. The main effects of probiotics on the immune system in different life stages in humans have been extensively been studied and targeted for these specific age groups (Romeo et al. 2010). Probiotic bacteria induce multiple effects on the host by changing the intestinal luminal environment, epithelial and mucosal barrier function and the mucosal immune system. Numerous cell types are involved in these responses, including the epithelial cells, dendritic cells (DC), monocytes/macrophages, B cells, T cells and NK cells (Ng et al. 2009). In murine models, several strains of lactobacilli enhance both the innate and adaptive immune response through induction of dendritic cell maturation and further stimulate the lymphocytes to release pro-inflammatory cytokines, including tumour necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin-12 (IL-12) (Perdigon et al. 1999).

The possible time-dependent role of LAB in immunomodulation was investigated in BALB/c mice receiving a daily diet, including *L. paracasei* subsp. *paracasei* NTU 101 (10^8 CFU/day) for 3, 6 and 9 weeks and following feeding with *Lactobacillus*-free food for a further 7 days (Tsai et al. 2008). The antigen-presenting ability of DC and the expression of natural killer group-2 D molecules capable of triggering natural killer cell-mediated cytotoxicity by feeding NTU 101 were found to be upregulated. Lymphocyte proliferation and antibody production were also significantly increased in mice after this treatment. Innate and adaptive immunity remained constant even after the most protracted feeding time. This indicates the time dependence of the bacterial-mediated enhanced immunity (Tsai et al. 2008). After feeding *Lactobacillus*, the percentages of $CD4^+$ T cells in both Peyer's patches and the spleen were significantly increased (Tsai et al. 2010a). The data also showed that Peyer's patch-derived immunomodulation induced higher levels of intestinal IgA^+-producing cells in the lamina propria. Feeding *Lactobacillus* also induced stronger $CD4^+$ T cell–dendritic cell interactions, enhanced $CD4^+$ T cell and B cell proliferation and increased mRNA expression levels of IL-1β, IL-10, IL-12, IFN-γ and TNF-α in Peyer's patches (Tsai et al. 2010a). It was also reported that intestinal DC could selectively induce IgA from the interactions with B cells and protect against the penetration of gut microorganisms in mucosal (Macpherson and Uhr 2004).

Chiang et al. (2011b) have reported immunomodulation effects of dead lactobacilli, whole cells and gastrointestinal enzymatic hydrolysates of supernatants and precipitates from *L. paracasei* subsp. *paracasei* NTU 101 on RAW264.7 macrophages and splenocytes. All of the hydrolysates and whole cells from NTU 101 were observed to induce lower levels of pro-inflammatory cytokines (IL-1β and IL-6) than LPS. The supernatant of NTU 101 was found to activate the cell division of RAW 264.7 macrophage from G0/G1 to the S phase and to facilitate the advance to the G2/M phase as well as increase the amount of IFN-γ in splenocytes, relative to the NTU 101 precipitates. This indicates that hydrolysates of NTU 101 induce the proliferation of macrophages and splenocytes and promote the release of IL-10 and IL-12 cytokines to modulate the innate and adaptive immune systems and inflammatory responses.

Polysaccharides are broadly used in the food industry as gelling, thickening, stabilizing and emulsifying agents. Certain probiotics such as LAB also produce exopolysaccharides that stimulate macrophage production of cytokines. Liu et al. (2011a) have characterised the effects of exopolysaccharides of *L. paracasei* subsp. *paracasei* NTU 101 exopolysaccharides (101EP) on antioxidant activity and immunomodulation in vitro. Cytokine production (including IL-6, TNF-α and IL-1β) was found to be induced by 101EP in Raw 264.7 cells in a dose-dependent manner. This also demonstrates the potential antioxidant properties (1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, chelating of ferrous ions, inhibition of linoleic acid peroxidation and reducing power) of 101EP in vitro (Liu et al. 2011a).

The health-promoting effects of probiotic strains or related fermented products are approved not only from themselves but also from their metabolites. According to these results of the immunomodulating potential, *L. paracasei* subsp. *paracasei* NTU 101 could enhance the lymphocyte proliferation and antibody production as well as promote the release of cytokines in intestinal tract by intact cells, hydrolysates or metabolites.

Prevention or alleviation of allergies and atopic diseases

Allergies are caused by an immune reaction that is disproportionate to the antigenic stimuli. A major proportion of the world population suffers from various types of allergies, and the incidence of allergies is on the rise (Ouwehand 2007). Pollen and food allergies and atopic dermatitis are classified as IgE-mediated allergic diseases. These diseases are major public health problems in many countries and are associated with elevated levels of antigen-specific IgE antibodies caused by improperly self-possessed T-helper (Th) type 2 responses (Yoshida et al. 2011).

Many types of LAB products, including dead or living bacterial cells and fermented foods, have been investigated for their anti-allergic effects in a murine model. Fujiwara et al. (2004) showed that orally administered *L. paracasei* KW3110 in a mouse allergy model directs the Th1/Th2 balance toward Th1 through the maturation of antigen-presenting cells and inhibition of serum IgE elevation. Sashihara et al. (2006) revealed that certain strains of heat-killed *L. plantarum* and *Lactobacillus gasseri* had a higher stimulatory activity for IL-12 production than the other lactobacilli tested. These species were also found to reduce the serum antigen-specific IgE levels in ovalbumin-sensitised BALB/c mice. Enomoto et al. (2009) showed that oral administration of *L. plantarum* NRIC0380 inhibits antigen-specific IgE production and enhances the Th1 response. In addition, orally administered heat-killed *Lactobacillus pentosus* strain S-PT84 has been shown to lower serum IgE levels, to suppress the active cutaneous anaphylaxis reaction and splenic IL-4 production and to upregulate IL-10 production in OVA-immunised mice (Nonaka et al. 2008).

It has also been shown that the allergic response may be induced by intraperitoneal injection or by oral or nasal administration of OVA in mice (Bae et al. 2007; Kim et al. 2008a; Medeiros et al. 2008; Hougee et al. 2010; Tobita et al. 2010a, b). In a previous study, 3-month-old male and female BALB/c mice were divided into two groups: a pre-sensitised group and a post-sensitised group. Levels of cytokines (IFN-γ, IL-4, IL-10 and IL-12), serum antibodies and subpopulations of lymphocytes were determined (Chiang 2011). The results indicate that pre-sensitised BALB/c mice have a higher level of allergy-related antibodies in the serum and an equal amount of cytokines derived from splenocytes in all groups. The oral administration of lactobacilli was found to reduce only the total IgE content in mice. For the post-sensitised model, the mice were fed lactobacilli before induction of the allergic response. As expected, the OVA-specific antibody levels were low after the first week. At the end of the test, orally administered *L. paracasei* subsp. *paracasei* NTU 101 showed an anti-allergic effect that arises as a result of a reduction in OVA-specific IgE levels, an increase in Th1-type cytokine IFN-γ levels and an increase in the IFN-γ/IL4 ratio. This treatment also induces lymphoproliferation activity as evidenced by the increase in the number of total T cell, T helper and T cytotoxic cells in the spleen. *L. casei* strain Shirota inhibited serum OVA-specific IgE and IgG1 responses and also skewed the pattern of cytokine secretion by splenocytes (Shida et al. 2002). Intragastric administration of these lactobacilli strains to mice would affect the cytokine Th1/Th2 balance towards non-allergic Th1 pathway. It also reduced the allergy-related antibodies in serum and induced the lymphocytes proliferation. These results indicate that the lactobacilli strains represent a potential application in therapy of allergic diseases.

Prevention of pathogen infection and modification of the intestinal microflora

The mechanism of involvement of probiotics in intestinal homeostasis has been investigated. Targets of probiotics have been found to be involved in the regulation of fluid movement, lactose digestion/absorption, improvement of the epithelial barrier function and increases in secretion of IgA (Ménard and Heyman 2006). Effects of probiotic *Lactobacillus* on the intestinal microflora and fermentation include inhibition of adherence and growth of pathogens and harmful bacteria by secretion of lactate and short chain fatty acids to decrease the luminal pH and stimulation of the colonic blood flow and epithelial cell growth (Ohashi and Ushida 2009).

Tsai et al. (2008) investigated the correlation of intestinal microflora with immunity induced by *L. paracasei* subsp. *paracasei* NTU 101. This correlation was determined by measuring the content of probiotics and harmful microorganisms. The results indicated alterations in intestinal microflora, with increased numbers of bifidobacteria and lactobacilli and decreased numbers of *Clostridium perfringens* after feeding with *L. paracasei* subsp. *paracasei* NTU 101. It is possible that persistent activation of immunity might be induced by intestinal probiotics (Tsai et al. 2008). Probiotics are also used as remedies for different types of intestinal infection diseases, such as traveller's diarrhoea caused by enterotoxigenic *Escherichia coli* (Caridi 2002; Ishida-Fujii et al. 2004; Sun et al. 2010), irritable bowel syndrome (Søndergaard et al. 2011) and enteric virus (Maragkoudakis et al. 2010). The barrier effect of intestinal bacteria against the challenge of pathogens is considered as its protective function.

E. coli O157:H7 is a highly infectious pathogen that causes food-borne illnesses (Karch et al. 2005). Infection often leads to haemorrhagic diarrhoea and kidney failure in young children and elderly individuals. In a previous study, the immunomodulating activity of *L. paracasei* subsp. *paracasei* NTU 101 was investigated in enterohaemorrhagic *E. coli* O157:H7-infected BALB/c mice (Tsai et al. 2010b). Mice were fed *L. paracasei* subsp. *paracasei* NTU 101 (10^8 CFU/day) for 7 days, before and after the challenge with *E. coli* O157:H7. Feeding of *Lactobacillus* for 7 days resulted in upregulation of dendritic cells, helper T cell activation and antibody production in post- and pre-treated mice, relative to untreated mice in the *E. coli* O157:H7-infected group. Moreover, *Lactobacillus* can decrease the expression of Toll-like receptors on macrophages and proinflammatory cytokines and chemokines in the post- or pre-fed mice infected with *E. coli* O157:H7. These findings suggest that *L. paracasei* subsp. *paracasei* NTU 101 might prevent human *E. coli* O157:H7 infections (Tsai et al. 2010b).

Common bacterial pathogens that cause gastrointestinal infection diarrhoea include *Campylobacter jejuni*, *Salmonella* and *Shigella* species, *E. coli* O157:H7 and *Helicobacter pylori* (Castillo et al. 2011; Ivanova et al. 2010; Moran 2010; Tsai et al. 2010b). Conventional treatment of bacterial infection is treated with antibiotics. To reduce the adverse effects of antibiotic therapy, continuous administration of lactobacilli probiotics may be a useful tool to protect against the pathogen infection. The protective mechanisms of probiotics from these studies are mainly proposed from modulating the inflammatory response against the enteropathogen in the gut.

Preventive effect on gastric mucosal lesions

Characteristics of ethanol-induced gastric mucosal injury include reductions in gastric mucus, motility, transmucosal potential difference, prostaglandin levels and gastric mucosal blood flow and increases in free radical generation, acid back diffusion, histamine levels, 5-hydroxytryptamine release, cation efflux, leukotriene production, ischemia and gastric vascular permeability (Glavin and Szabo 1992).

Many reports suggest that LAB and their fermented products have protective effects against mucosal injury in the stomach. Yogurt-containing *L. gasseri* OLL2716 (LG21 yogurt), which can compete with *H. pylori* in the gastric mucus layer, resulting in decreased colonisation of the organism (Fujimura et al. 2006), has been shown to be effective in improving *H. pylori*-induced gastric mucosal inflammation in humans (Sakamoto et al. 2001). In oral administration of *L. casei* strain Shirota in *H. pylori* SS1-infected mice, levels of *H. pylori* colonisation were significant reduction in the antrum and body mucosa. It also observed that the associated chronic and active gastric mucosal inflammation was decline (Sgouras et al. 2004).

Uchida and Kurakazu (2004) reported that the ingestion of LG21 yogurt significantly and dose-dependently inhibits the formation of acute gastric lesions caused by 0.6 M HCl in rats. LG21 was also observed to increase the rate of prostaglandin E2 (PGE2) generation in the gastric mucosa, which leads to prevention of gastric ulcers. *Lactobacillus rhamnosus* GG has also been reported to enhance gastric healing by activation of epidermal growth factor receptor and upregulation of expression of ornithine decarboxylase, B cell lymphoma 2 and vascular endothelial growth factor in ulcerated tissues (Lam et al. 2007a). *L. rhamnosus* GG has also been reported to stimulate mucus secretion and to increase transmucosal resistance in the gastric mucosa (Lam et al. 2007b).

In a previous study, *L. paracasei* subsp. *paracasei* NTU 101 and *L. plantarum* NTU 102 were used as starter to ferment soy–skim milk. The influence of LAB-fermented soy–skim milk on mucosal integrity in a gastric mucosal lesion rat model was investigated (Liu et al. 2009). Pylorus ligation with acidified ethanol treatment was used as a gastric lesion animal model. Results showed that LAB-fermented soy–skim milk could significantly reduce the lipid peroxides of gastric mucosa and serum and the gastric lesion index (Fig. 4 and Table 1). Dose-dependent inhibition of acute gastric lesions was observed for the NTU 101 (10^9 CFU/day, 101-9 group) with significant differences. The high dose of fermented soy–skim milk was also found to enhance superoxide dismutase activity and PGE2 synthesis. Rats fed commercial yogurt and soy–skim milk fermented by NTU 101 (101-9) had normal mucosal structure, which was significantly different from the mucosal structure of the rats that were fed water or non-fermented soy–skim milk. This normal mucosal structure is expected to protect the rats against gastric injury.

From these results mentioned above, lactobacilli strains may possess the defence against the injuries caused from *H. pylori* infection or acidified ethanol gastric lesion.

Anti-osteoporosis effect

Osteoporosis, a major skeletal disease associated with aging, has a number of subtypes, including senile osteoporosis and postmenopausal or menopause-related osteoporosis (Pietschmann et al. 2009). Osteoporosis affects great numbers of individuals of both sexes and all races. Its prevalence increases with age, and it has been estimated that more than ten million Americans have osteoporosis and an additional

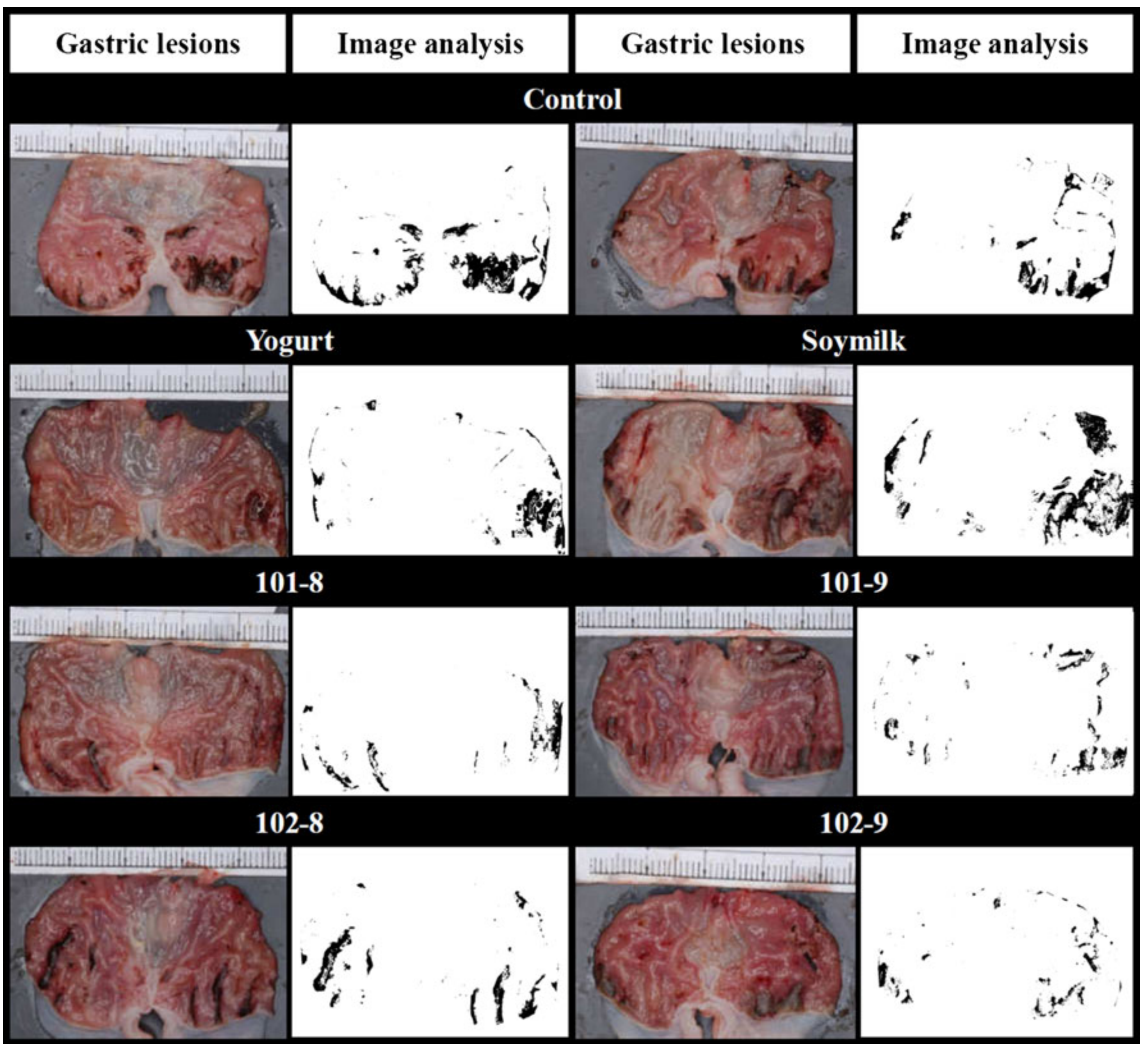


Fig. 4 Image analysis of gastric lesion. Rats fed yogurt, soy–skim milk and fermented soy–skim milk for 4 weeks after pylorus ligation combined with acidified ethanol induced acute gastric lesions of gastric mucosa (*left*); the same images after optimised background subtraction by ImageJ software (*right*) (Liu et al. 2009)

Table 1 Effects of gastric lesion index in rats

Group	Gastric lesion index		
	Lesion area (mm^2)	Total mucosal area (mm^2)	Lesion index
Control	99.01±18.43a	977.99±65.14a	1.01±0.18a
Yogurt	70.02±12.14ab	991.75±40.01a	0.70±0.12ab
Soy–skim milk	98.95±6.52a	1,010.33±46.62a	0.99±0.06a
101-8	84.83±12.47ab	1,054.08±43.85a	0.80±0.11ab
101-9	53.26±8.13b	993.42±45.77a	0.55±0.09b
102-8	72.89±13.36ab	998.60±39.74a	0.72±0.12ab
102-9	79.33±15.98ab	1,098.40±26.40a	0.66±0.14ab

Data are presented as mean (SEM) (n=8); numbers with different letters within the same column are significantly different (p<0.05) (Liu et al. 2009)

Control normal diet, *Yogurt* commercial yogurt diet, *Soy–skim milk* nonfermented soy–skim milk, *101-8* 10^8 CFU/day of soy–skim milk fermented by *L. paracasei* subsp. *paracasei* NTU 101, *101-9* 10^9 CFU/day of soy–skim milk fermented by *L. paracasei* subsp. *paracasei* NTU 101, *102-8* 10^8 CFU/day of soy–skim milk fermented by *L. plantarum* NTU 102; *102-9* 10^9 CFU/day of soy–skim milk fermented by *L. plantarum* NTU 102

33.6 million have low bone density of the hip (NOF 2010). Loss of oestrogen seems to be the most important mechanism in the development of osteoporosis. The use of ovariectomised (OVX) rat or mice models, a well-established and reproducible method of stimulating the postmenopausal condition, has been found to be effective in mimicking postmenopausal cancellous bone loss (Egermann et al. 2005).

In a previous study, *L. paracasei* subsp. *paracasei* NTU 101 and *L. plantarum* NTU 102 were used as starter to ferment soy–skim milk (NTU 101F and NTU 102F) and then used as a nutritional supplement for 8 weeks in OVX mice (Chiang and Pan 2011). The results showed that soy–skim milk fermented with lactobacilli can increase the content of aglycone isoflavones, soluble calcium and vitamin D_3. The trabecular bone volumes and trabecular number of the distal femur in mice fed NTU 101F increased by factors of 1.48 and 1.74, respectively, relative to the OVX group. The 3D reconstructions of micro-CT imaging and SEM images (×80 and ×130) of the endosteal surfaces of the femur metaphysis at the ultrastructural level are shown in Fig. 5. The growth plate in sham group was thicker than that in the other OVX groups and showed that the sham mice were still growing. The images of trabecular bone in the sham and OVX+NTU 101F groups indicate significantly greater thickness and more plate formation than the images of the OVX group in thinner rod form. The bone network density and thickness of the distal metaphyseal trabecular bone in mice fed NTU 101F was significantly greater than that of the OVX mice.

Physiological phenomena associated with aging include post-maturation processes, diminished homeostatic capacity, reduced responsiveness to stimuli (Candore et al. 2008), impaired memory acquisition, decreased immune responses, increased peroxidation in vivo and loss of bone density (Hayflick 2007; Candore et al. 2006). Chiang et al. (2011a) also used lactobacilli-fermented soy–skim milk as a nutritional supplement for 6 weeks to investigate its anti-osteoporosis effect in 13-month-old female BALB/c (aging) mice. In particular, the content of polysaccharides and peptides of the NTU 101-fermented milk was the highest among the groups (4.5 and 335.2 mg/g, respectively). The trabecular bone volumes in mice fed NTU 101F increased by a factor of 3.48 relative to controls. The network density and thickness of distal metaphyseal trabecular bone in mice fed NTU 101F milk was significantly higher than those in the control mice. Moreover, the NTU 101F group had the largest resting area ratio (84.5±4.5%) and the smallest resorbing area (12.1±4.2%) when compared with the other groups in Fig. 6. It was not possible to produce data for the forming area for all aging mice as a result of the bone remodelling rate and the decrease in bone mass with increased age. The combined results of the OVX and aging models suggest that dietary supplementation with NTU-101 fermented soy–skim milk can attenuate bone loss in mice and possibly lower the risk of osteopenia or osteoporosis (Chiang et al. 2011a).

The effect of long-term feeding of milk fermented with *L. helveticus* on bone in growing rats was evaluated in SHR rats (Narva et al. 2004). The bone mineral content and bone mineral density related to the weight of the rats were the highest after feeding with the *L. helveticus* fermented milk, which contained more amounts of the IPP and VPP peptides. Compared with the results of NTU 101F, it showed that the fermentation process would enhance the production of bioactive components and reveal the anti-osteoporosis activity. Further studies are needed to confirm and clarify the mechanism of lactobacilli-fermented products.

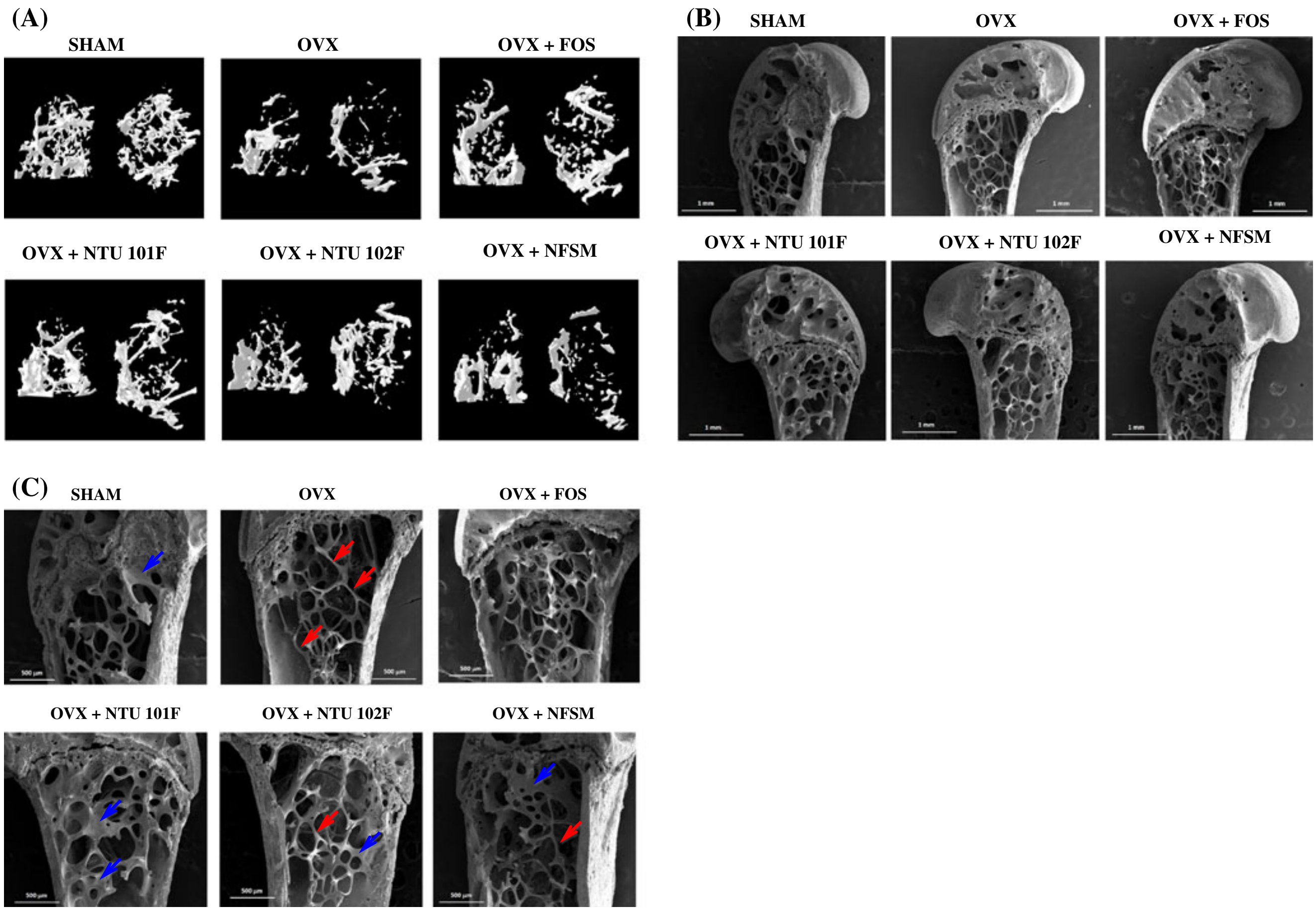


Fig. 5 Microstructural properties of the distal femoral metaphyseal trabecular bone in C57BL/6J female ovariectomised mice after 8 weeks of oral administration of either *L. paracasei* subsp. *paracasei* NTU 101 or *L. plantarum* NTU 102-fermented and nonfermented soy–skim milk. **a** Three-dimensional tomographic rendering of a mouse femur revealing the complexity of the bone structure (images of the distal femoral metaphysis trabecular bone in 1.5-mm-thick images; the *left-hand image* shows the side view and the *right-hand image* the top view); **b** Electron microphotographs of the endosteal surface in the femoral metaphysis of OVX C57BL/6J mice at ×80; **c** ×130. *Scale bar*: **b** 1 mm; **c** 500 μm. *Red arrow* represents the cylindrical rod form trabecular bone, and the *blue arrow* represents the parallel plate form trabecular bone. (Chiang and Pan 2011)

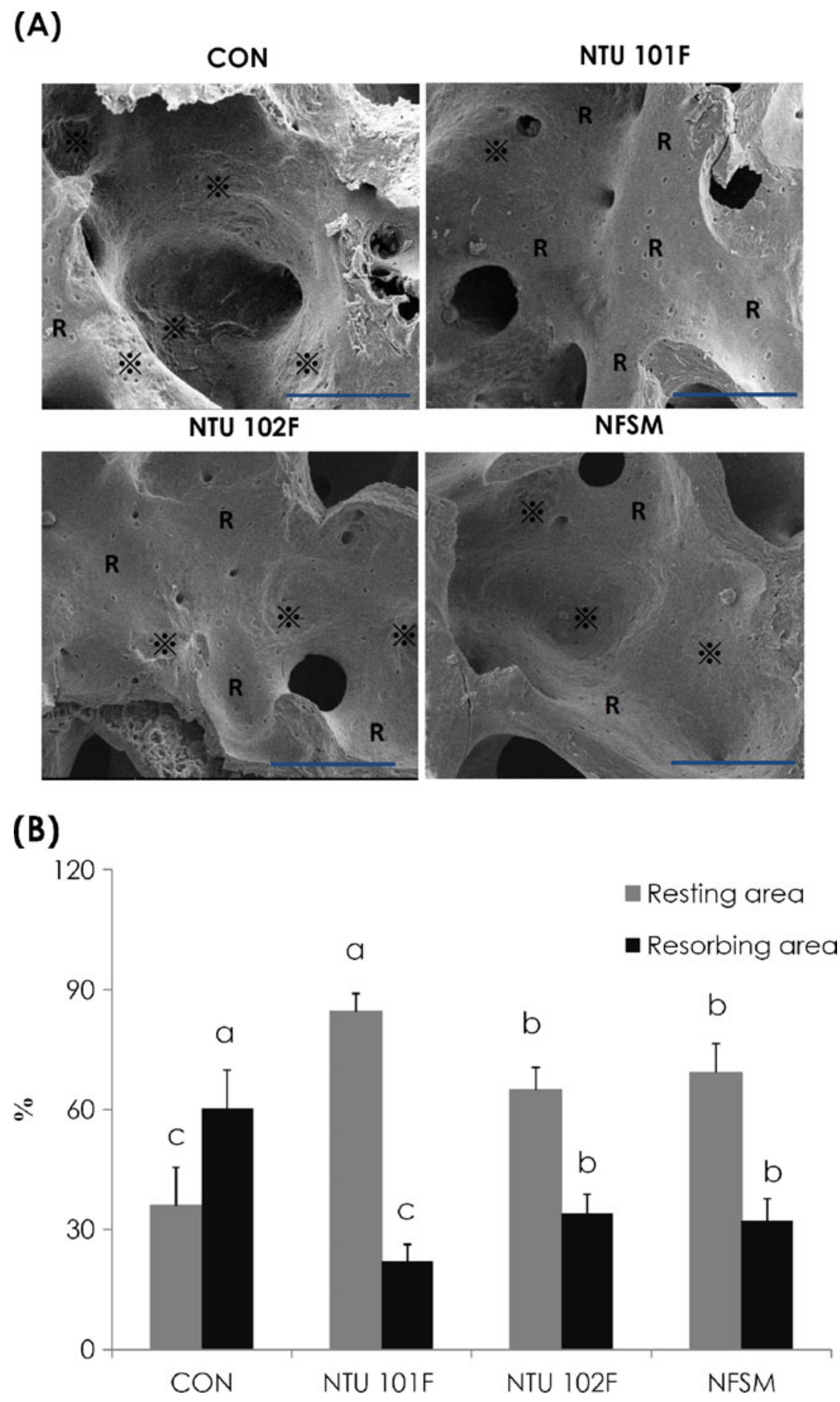
Prevention of fat accumulation

Obesity is becoming a global epidemic in both children and adults and is associated with morbidity due to CVDs, type II diabetes, hypertension, metabolic syndrome and certain cancers (Poirier et al. 2006). These forms of obesity have two important characteristics: increased accumulation of adipose tissue and secretion of pathogenetic products from enlarged fat cells (Bray 2004). The prevalence of obesity was 32.2% among adult men and 35.5% among adult women from 2007 to 2008 in the USA. Increases in the outcomes of obesity have previously been observed and exceeding 30% in most sex and age groups (Flegal et al. 2010).

The composition of the microbial community is inherited from mothers and is similar at the division level in mice and humans. In obese mice vs. lean mice, the abundance of *Bacteroidetes* decreases to 50% and indicates that obesity affects the diversity of the gut microbiota (Ley et al. 2005). Cani and Delzenne (2009) hypothesised that relationships between the colon and the regulation of energy metabolism are influenced by calories ingested, synthesis of gut peptides and fat storage. Moreover, the development of obesity and metabolic disorders as a result of a high-fat diet may be associated with an imbalance in the innate immune system, development of metabolic endotoxemia and atherogenic markers in subjects (Cani and Delzenne 2009).

Many recent studies have focused on the anti-obesity effects of LAB (mainly the bifidobacteria and *Lactobacillus* strains). Choi et al. (2007) indicated that oral administration of *Bifidobacterium breve*-fermented isoflavone aglycones effectively suppresses absorption of excessive lipid into the body and inhibits adipocyte differentiation in 3T3-L1 cells in a dose-dependent manner. Mice fed a high-fat diet supplemented with *B. breve* B-3 exhibit dose-dependent suppression of accumulation of epididymal fat and

Fig. 6 Measurements of endosteal surface of femoral diaphysis in aging BALB/c mice. **a** Scanning electron microphotographs of the endosteal surface in the femoral diaphysis in aging BALB/c mice. **b** Percentage of bone surface types on the endosteal surface of the femoral diaphysis. *Scale bar* 200 μm. *R* resting area, *asterisk* resorbing area. Data are expressed as mean±SD (n=5). Means with *different letters* compared within the resorbing area or resting area were significantly different (p<0.01) (Chiang et al. 2011a)



upregulation of genes related to fat metabolism and insulin sensitivity in the gut and epididymal fat tissue (Kondo et al. 2010). Kim et al. (2008b) showed that the Soypro™ (a new brand of soymilk) fermented by *Leuconostoc citreum* and *L. plantarum* decreases levels of LDL-C in SD rats and inhibits the differentiation of 3T3-L1 adipocytes (Kim et al. 2008a, b). *L. plantarum* strain no. 14 was also reported to decrease the mean adipocyte size and white adipose tissue weight and to decrease the serum total cholesterol and leptin concentrations (Takemura et al. 2010). The anti-obesity effects of *L. gasseri* SBT2055 cause lowering of abdominal adiposity, body weight, body mass index and waist and hip circumference. These observations suggest that this strain exerts a beneficial influence on metabolic disorders (Kadooka et al. 2010). In contrast, *L. rhamnosus* PL60 was found to only reduce the body weight and white (epididymal and perirenal) adipose tissue due to production of t10, c12-conjugated linoleic acid (Lee et al. 2006).

The flavonoid genistein was found to inhibit proliferation of preconfluent preadipocytes in a time- and dose-dependent manner and to regulate body fat in terms of preadipocyte replication, differentiation and lipolysis (Harmon and Harp 2001). Daidzein is another flavonoid that can significantly reduce body fat weight and ameliorate hyperlipidemia. This flavonoid also inhibits the activity of pancreatic and lipoprotein lipase and the differentiation of rat preadipocytes (Guo et al. 2009).

In a previous study, Lo (2011) reported that the *L. paracasei* subsp. *paracase*i NTU 101 (NTU 101) strain and its fermented soy–skim milk supernatant (SM 101) administered as supplements for 5 weeks produce anti-obesity effects in rats maintained on a high-fat diet. A high-fat diet supplemented with NTU 101 and SM101 can significantly decrease feed efficiency, body weight and body fat pad weight of rats. SM101 causes decreases in feed efficiency, body weight and body fat pad weight of 53.2%, 49.7% and 55.9%, respectively. Moreover, NTU 101 and SM101 also reduce the average radius of adipocytes and increase the number of small adipocytes. These results reveal that NTU 101 and SM 101 have the ability to prevent the body fat accumulation. The bioactive compounds in NTU 101 and SM 101 that possess the prevention effect in fat accumulation may be the aglycone isoflavones.

Regardless of the cell line system or diet-induced obesity animal model used, these results were showed that LAB inhibited the adipocyte differentiation and reduced the body weight gain, white adipose tissue weight and mean adipocyte size.

Conclusion

Many pioneering scientific studies on beneficial effects of *L. paracasei* subsp. *paracase*i NTU 101 and its fermented products have been conducted by different methodologies and test system trials. The evidences on this probiotic strain are performed to ameliorate hypercholesterolemia, hypertension, allergy, gastric lesion, osteoporosis and obesity. The production of *Lactobacillus*-fermented products is improved to enhance the contents of GABA, biogenic peptides and aglycone isoflavones. Therefore, more data will be forthcoming to explore its bioactive components and their therapeutic effects in the near future.

References

Ahrne S, Nobaek S, Jeppsson B, Adlerberth I, Wold AE, Molin G (1998) The normal *Lactobacillus* flora of healthy human rectal and oral mucosa. J Appl Microbiol 85:88–94

Bae EA, Min SW, Lee B, Kim NJ, Baek NI, Han EJ, Chung HG, Kim DH (2007) Antiasthmic effect of fermented *Artemisia princeps* in asthmic mice induced by ovalbumin. J Microbiol Biotechnol 17:1554–1557

Bray G (2004) A medical consequences of obesity. J Clin Endocrinol Metab 89:2583–2589

Candore G, Colonna-Romano G, Balistreri CR, Di Carlo D, Grimaldi MP, Listì F, Nuzzo D, Vasto S, Lio D, Caruso C (2006) Biology of longevity: role of the innate immune system. Rejuv Res 9:143–148

Candore G, Balistreri CR, Colonna-Romano G, Grimaldi MP, Lio D, Listì F, Scola L, Vasto S, Caruso C (2008) Immunosenescence and anti-immunosenescence therapies: the case of probiotics. Rejuv Res 11:425–432

Cani PD, Delzenne NM (2009) The role of the gut microbiota in energy metabolism and metabolic disease. Curr Pharm Des 15:1546–1558

Caridi A (2002) Selection of *Escherichia coli*-inhibiting strains of *Lactobacillus paracasei* subsp. *paracasei*. J Ind Microbiol Biotechnol 29:303–308

Castillo NA, Perdigón G, de Moreno de Leblanc A (2011) Oral administration of a probiotic *Lactobacillus* modulates cytokine production and TLR expression improving the immune response against *Salmonella enterica* serovar Typhimurium infection in mice. BMC Microbiol 11:177

Chiang SS (2011) The allergenicity assessment of *Lactococcus lactis* NZ9000/pNZPNK and evaluation of the probiotic effects on *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and *Lactobacillus plantarum* NTU 102. Ph.D. dissertation, National Taiwan University, Taipei, Taiwan

Chiang SS, Pan TM (2011) Antiosteoporotic effects of lactobacillus-fermented soy skim milk on bone mineral density and the microstructure of femoral bone in ovariectomised mice. J Agric Food Chem 59:7734–7742

Chiang SS, Liao JW, Pan TM (2011a) Effect of bioactive compounds in lactobacilli-fermented soy skim milk on femoral bone microstructure of aging mice. J Sci Food Agric. doi:10.1002/jsfa.4579

Chiang SS, Liu CF, Tseng KC, Mau JL, Pan TM (2011b) Immunomodulatory effects of dead *Lactobacillus* on murine splenocytes and macrophages. Food Agr Immunol. doi:10.1080/09540105.2011.609246

Chiu CH, Lu TY, Tseng YY, Pan TM (2006) The effects of *Lactobacillus*-fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet. Appl Microbiol Biotechnol 71:238–245

Choi I, Kim Y, Park Y, Seog H, Choi H (2007) Anti-obesity activities of fermented soygerm isoflavones by *Bifidobacterium breve*. Biofactors 29:105–112

de Vrese M, Schrezenmeir J (2008) Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol 111:1–66

Egermann M, Goldhahn J, Schneider E (2005) Animal models for fracture treatment in osteoporosis. Osteoporosis Int 16:S129–S138

Enomoto M, Noguchi S, Hattori M, Sugiyama H, Suzuki Y, Hanaoka A, Okada S, Yoshida T (2009) Oral administration of *Lactobacillus plantarum* NRIC0380 suppresses IgE production and induces $CD4^{(+)}CD25^{(+)}Foxp3^{(+)}$ cells *in vivo*. Biosci Biotechnol Biochem 73:457–460

Flegal KM, Carroll MD, Ogden CL, Curtin LR (2010) Prevalence and trends in obesity among US adults, 1999–2008. JAMA 303:235–241

Fooks LJ, Fuller R, Gibson GR (1999) Prebiotics, probiotics and human gut microbiology. Int Dairy J 9:53–61

Fuglsang A, Nilsson D, Nyborg NC (2002) Cardiovascular effects of fermented milk containing angiotensin-converting enzyme inhibitors evaluated in permanently catheterised, spontaneously hypertensive rats. Appl Environ Microbiol 68:3566–3569

Fujimura S, Kato S, Oda M, Miyahara M, Ito Y, Kimura K, Kawamura T, Ohnuma M, Tateno H, Watanabe A (2006) Detection of *Lactobacillus gasseri* OLL2716 strain administered with yogurt drink in gastric mucus layer in humans. Lett Appl Microbiol 43:578–581

Fujiwara D, Inoue S, Wakabayashi H, Fujii T (2004) The anti-allergic effects of lactic acid bacteria are strain dependent and mediated by effects on both Th1/Th2 cytokine expression and balance. Int Arch Allergy Immunol 135:205–215

Fukushima M, Nakano M (1996) Effects of mixture of organisms, *Lactobacillus acidophilus* or *Streptococcus faecalis* on cholesterol metabolism in rats fed on a fat- and cholesterol-enriched diet. Br J Nutr 76:857–867

Glavin GB, Szabo S (1992) Experimental gastric mucosal injury: laboratory models reveal mechanisms of pathogenesis and new therapeutic strategies. FASEB J 6:825–831

Guo Y, Wu G, Su X, Yang H, Zhang J (2009) Antiobesity action of a daidzein derivative on male obese mice induced by a high-fat diet. Nutrition Res 29:656–663

Harmon AW, Harp JB (2001) Differential effects of flavonoids on 3T3-L1 adipogenesis and lipolysis. Am J Physiol Cell Physiol 280:807–813

Hata Y, Yamamoto M, Ohni M, Nakajima K, Nakamura Y, Takano T (1996) A placebo-controlled study of the effect of sour milk on blood pressure in hypertensive subjects. Am J Clin Nutr 64:767–771

Hayflick L (2007) Biological aging is no longer an unsolved problem. Ann NY Acad Sci 1100:1–13

Holzapfel WH (2006) Introduction to prebiotics and probiotics. In: Goktepe et al (eds) Probiotics in food safety and human health. CRC (Taylor & Francis Group), Boca Raton, pp 1–33

Hougee S, Vriesema AJ, Wijering SC, Knippels LM, Folkerts G, Nijkamp FP, Knol J, Garssen J (2010) Oral treatment with probiotics reduces allergic symptoms in ovalbumin-sensitised mice: a bacterial strain comparative study. Int Arch Allergy Immunol 151:107–117

Imig JD (2004) ACE inhibition and bradykinin-mediated renal vascular responses: EDHF involvement. Hypertension 43:533–535

Ishida-Fujii K, Goto S, Kuboki H, Hirano S, Sakamoto M, Sato M (2004) Isolation and identification of lactic acid bacteria with effect of immune protection to *Escherichia coli* in mice. Biofactors 21:155–158

Ivanova K, Marina M, Petrov P, Kantardjiev T (2010) Campylobacteriosis and other bacterial gastrointestinal diseases in Sofia, Bulgaria for the period 1987–2008. Euro Surveill 15:19474

Jauhiainen T, Korpela R (2007) Milk peptides and blood pressure. J Nutr 137:825S–829S

Jauhiainen T, Rönnback M, Vapaatalo H, Wuolle K, Kautiainen H, Groop PH, Korpela R (2010) Long-term intervention with *Lactobacillus helveticus* fermented milk reduces augmentation index in hypertensive subjects. Eur J Clin Nutr 64:424–431

Kadooka Y, Sato M, Imaizumi K, Ogawa A, Ikuyama K, Akai Y, Okano M, Kagoshima M, Tsuchida T (2010) Regulation of abdominal adiposity by probiotics (*Lactobacillus gasseri* SBT2055) in adults with obese tendencies in a randomised controlled trial. Eur J Clin Nutr 64:636–643

Kapila S, Sinha PR (2006) Antioxidative and hypocholesterolemic effect of *Lactobacillus casei* subsp *casei* (biodefensive properties of lactobacilli). Indian J Med Sci 60:361–370

Karch H, Tarr P, Bielaszewska M (2005) Enterohaemorrhagic *Escherichia coli* in human medicine. Int J Med Microbiol 295:405–418

Kim JY, Choil YO, Ji GE (2008a) Effect of oral probiotics (*Bifidobacterium lactis* AD011 and *Lactobacillus acidophilu*s AD031) administration on ovalbumin-induced food allergy mouse model. J Microbiol Biotechnol 18:1393–1400

Kim NH, Moon PD, Kim SJ, Choi IY, An HJ, Myung NY, Jeong HJ, Um JY, Hong SH, Kim HM (2008b) Lipid profile lowering effect of Soypro™ fermented with lactic acid bacteria isolated from Kimchi in high-fat diet-induced obese rats. Biofactors 33:49–60

Kondo S, Xiao JZ, Satoh T, Odamaki T, Takahashi S, Sugahara H, Yaeshima T, Iwatsuki K, Kamei A, Abe K (2010) Antiobesity effects of *Bifidobacterium breve* strain B-3 supplementation in a mouse model with high-fat diet-induced obesity. Biosci Biotechnol Biochem 74:1656–1661

Lam EK, Tai EK, Koo MW, Wong HP, Wu WK, Yu L, So WH, Woo PC, Cho CH (2007a) Enhancement of gastric mucosal integrity by *Lactobacillus rhamnosus* GG. Life Sci 80:2128–2136

Lam EK, Yu L, Wong HP, Wu WK, Shin VY, Tai EK, So WH, Woo PC, Cho CH (2007b) Probiotic *Lactobacillus rhamnosus* GG enhances gastric ulcer healing in rats. Eur J Pharmacol 565:171–179

Lee HY, Park JH, Seok SH, Baek MW, Kim DJ, Lee KE, Paek KS, Lee Y, Park JH (2006) Human originated bacteria, *Lactobacillus rhamnosus* PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. Biochim Biophys Acta 1761:736–744

Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI (2005) Obesity alters gut microbial ecology. Proc Natl Acad Sci 102:11070–11075

Lin FM, Chiu CH, Pan TM (2004) Fermentation of a milk–soymilk and *Lycium chinense* miller mixture using a new isolate of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and *Bifidobacterium longum*. J Ind Microbiol Biotechnol 31:559–564

Liu CF, Hu CL, Chiang SS, Tseng KC, Yu RC, Pan TM (2009) Beneficial preventive effects of gastric mucosal lesion for soy–skim milk fermented by lactic acid bacteria. J Agric Food Chem 57:4433–4438

Liu CF, Tseng KC, Chiang SS, Lee BH, Hsu WH, Pan TM (2011a) Immunomodulatory and antioxidant potential of *Lactobacillus* exopolysaccharides. J Sci Food Agric 91:2284–2291

Liu CF, Tung YT, Wu CL, Lee BH, Hsu WH, Pan TM (2011b) Antihypertensive effects of *Lactobacillus*-fermented milk orally administered to spontaneously hypertensive rats. J Agric Food Chem 59:4537–4543

Lo YH (2011) The effect of *Lactobacillus paracasei* subsp. *paracase*i NTU 101 and *Lactobacillus plantarum* NTU 102 prevent the development of obesity and inflammatory response induced by high-fat diet. Master dissertation, National Taiwan University, Taipei, Taiwan

Macpherson AJ, Uhr T (2004) Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science 303:1662–1665

Maragkoudakis PA, Chingwaru W, Gradisnik L, Tsakalidou E, Cencic A (2010) Lactic acid bacteria efficiently protect human and animal intestinal epithelial and immune cells from enteric virus infection. Int J Food Microbiol 141:S91–S97

Medeiros KC, Figueiredo CA, Figueredo TB, Freire KR, Santos FA, Alcantara-Neves NM, Silva TM, Piuvezam MR (2008) Anti-allergic effect of bee pollen phenolic extract and myricetin in ovalbumin-sensitised mice. J Ethnopharmacol 119:41–46

Ménard S, Heyman M (2006) Modulation of epithelial function and local immune system by probiotics: mechanisms involved. In: Goktepe et al (eds) Probiotics in food safety and human health. CRC (Taylor & Francis Group), Boca Raton, pp 341–363

Moran AP (2010) The role of endotoxin in infection: *Helicobacter pylori* and *Campylobacter jejuni*. Subcell Biochem 53:209–240

Narva M, Collin M, Lamberg-Allardt C, Kärkkäinen M, Poussa T, Vapaatalo H, Korpela R (2004) Effects of long-term intervention with *Lactobacillus helveticus*-fermented milk on bone mineral density and bone mineral content in growing rats. Ann Nutr Metab 48:228–234

National Osteoporosis Foundation (NOF) (2010) Clinician's guide to prevention and treatment of osteoporosis. National Osteoporosis Foundation, Washington, DC

Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC (2009) Mechanisms of action of probiotics: recent advances. Inflamm Bowel Dis 15:300–310

Noh DO, Kim SH, Gilliland SE (1997) Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus* ATCC 43121. J Dairy Sci 80:3107–3113

Nonaka Y, Izumo T, Izumi F, Maekawa T, Shibata H, Nakano A, Kishi A, Akatani K, Kiso Y (2008) Antiallergic effects of *Lactobacillus pentosus* strain S-PT84 mediated by modulation of Th1/Th2 immunobalance and induction of IL-10 production. Int Arch Allergy Immunol 145:249–257

Ohashi Y, Ushida K (2009) Health-beneficial effects of probiotics: its mode of action. Anim Sci J 80:361–371

Ouwehand AC (2007) Antiallergic effects of probiotics. J Nutr 137:794S–797S

Pan TM, Chiu CH, Guu YK (2002) Characterization of *Lactobacillus* isolates from pickled vegetables for use as dietary or pickle adjuncts. Foods & Food Ingred J Jpn 206:45–51

Perdigon G, Vintini E, Alvarez S, Medina M, Medici M (1999) Study of the possible mechanisms involved in the mucosal immune system activation by lactic acid bacteria. J Dairy Sci 82:1108–1114

Pietschmann P, Rauner M, Sipos W, Kerschan-Schindl K (2009) Osteoporosis: an age-related and gender-specific disease—a mini-review. Gerontology 55:3–12

Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH (2006) Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. Arterioscler Thromb Vasc Biol 26:968–976

Romeo J, Nova E, Warnberg J, Gomez-Martinez S, Diaz Ligia LE, Marcos A (2010) Immunomodulatory effect of fibres, probiotics and synbiotics in different life-stages. Nutr Hosp 25:341–349

Sakamoto I, Igarashi M, Kimura K, Takagi A, Miwa T, Koga Y (2001) Suppressive effect of *Lactobacillus gasseri* OLL 2716 (LG21) on *Helicobacter pylori* infection in humans. J Antimicrob Chemother 47:709–710

Sashihara T, Sueki N, Ikegami S (2006) An analysis of the effectiveness of heat-killed lactic acid bacteria in alleviating allergic diseases. J Dairy Sci 89:2846–2855

Schmid K, Schlothauer RC, Friedrich U, Staudt C, Apajalahti J, Hansen EB (2006) Development of probiotic food ingredients. In: Goktepe et al (eds) Probiotics in food safety and human health. CRC (Taylor & Francis Group), Boca Raton, pp 35–66

Seppo L, Jauhiainen T, Poussa T, Korpela R (2003) A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. Am J Clin Nutr 77:326–330

Sgouras D, Maragkoudakis P, Petraki K, Martinez-Gonzalez B, Eriotou E, Michopoulos S, Kalantzopoulos G, Tsakalidou E, Mentis A (2004) In vitro and in vivo inhibition of *Helicobacter pylori* by *Lactobacillus casei* strain Shirota. Appl Environ Microbiol 70:518–526

Shida K, Takahashi R, Iwadate E, Takamizawa K, Yasui H, Sato T, Habu S, Hachimura S, Kaminogawa S (2002) *Lactobacillus casei* strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model. Clin Exp Allergy 32:563–570

Søndergaard B, Olsson J, Ohlson K, Svensson U, Bytzer P, Ekesbo R (2011) Effects of probiotic fermented milk on symptoms and intestinal flora in patients with irritable bowel syndrome: a randomised, placebo-controlled trial. Scand J Gastroenterol 46:663–672

Sun J, Hu XL, Le GW, Shi YH (2010) Lactobacilli prevent hydroxy radical production and inhibit *Escherichia coli* and *Enterococcus* growth in system mimicking colon fermentation. Lett Appl Microbiol 50:264–269

Takano T (2002) Anti-hypertensive activity of fermented dairy products containing biogenic peptides. Antonie Van Leeuwenhoek 82:333–340

Takemura N, Okubo T, Sonoyama K (2010) *Lactobacillus plantarum* strain No. 14 reduces adipocyte size in mice fed high-fat diet. Exp Biol Med (Maywood) 235:849–856

Tobita K, Yanaka H, Otani H (2010a) Anti-allergic effects of *Lactobacillus crispatus* KT-11 strain on ovalbumin-sensitised BALB/c mice. Anim Sci J 81:699–705

Tobita K, Yanaka H, Otani H (2010b) The antiallergic effects and acute toxicity of *Lactobacillus crispatus* KT-11 cultured in food grade medium. J Agric Food Chem 58:6498–6502

Tsai YT, Cheng PC, Fan CK, Pan TM (2008) Time-dependent persistence of enhanced immune response by a potential probiotic strain *Lactobacillus paracasei* subsp. *paracasei* NTU 101. Int J Food Microbiol 128:219–225

Tsai TY, Chu LH, Lee CL, Pan TM (2009) Atherosclerosis preventing activity of lactic acid bacteria-fermented milk–soymilk supplemented with *Momordica charantia*. J Agric Food Chem 57:2065–2071

Tsai YT, Cheng PC, Liao JW, Pan TM (2010a) Effect of the administration of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 on Peyer's patch-mediated mucosal immunity. Int Immunopharmacol 10:791–798

Tsai YT, Cheng PC, Pan TM (2010b) Immunomodulating activity of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 in enterohemorrhagic *Escherichia coli* O157:H7-infected mice. J Agric Food Chem 58:11265–11272

Uchida M, Kurakazu K (2004) Yogurt containing *Lactobacillus gasseri* OLL2716 exerts gastroprotective action against acute gastric lesion and antral ulcer in rats. J Pharmacol Sci 96:84–90

USDA (2011) Dairy products annual summary. National Agricultural Statistics Service (NASS), USDA, Office of the Assistant Secretary for Civil Rights, Washington, DC, pp 9–51

WHO (2011) The top 10 causes of death; Fact sheet N°310. WHO, Geneva. http://www.who.int/mediacentre/factsheets/fs310/en/index.html. Accessed 26 August 2011.

Xie N, Cui Y, Yin YN, Zhao X, Yang JW, Wang ZG, Fu N, Tang Y, Wang XH, Liu XW, Wang CL, Lu FG (2011) Effects of two *Lactobacillus* strains on lipid metabolism and intestinal microflora in rats fed a high-cholesterol diet. BMC Complement Altern Med 11:53–63

Yamamoto N, Maeno M, Takano T (1999) Purification and characterization of an antihypertensive peptide from a yogurt-like product fermented by *Lactobacillus helveticus* CPN4. J Dairy Sci 82:1388–1393

Yeo SK, Liong MT (2010) Angiotensin I-converting enzyme inhibitory activity and bioconversion of isoflavones by probiotics in soymilk supplemented with prebiotics. Int J Food Sci Nutr 61:161–181

Yoshida A, Aoki R, Kimoto-Nira H, Kobayashi M, Kawasumi T, Mizumachi K, Suzuki C (2011) Oral administration of live *Lactococcus lactis* C59 suppresses IgE antibody production in ovalbumin-sensitised mice via the regulation of interleukin-4 production. FEMS Immunol Med Microbiol 61:315–322